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Attn: 8(d) HEALTH & SAFETY STUDY REPORTING RULE
(REPORTING)

Dear Sir or Madam:

We herewith submit a copy of a study report that is being developed into a manuscript and will be submitted for publication. The International Isocyanate Institute (III) reference # is 11298. Please refer to the III identification number in any communication regarding this study. The enclosed report does not contain any Confidential Busines Information.

"Determination of the effect of TDI on the Reproduction of Daphnia Magna."

Name of Chemical Substance:	80% toluene 2,4-diisocyanate & 20% toluene 2,6-diisocyanate
Chemical Abstracts Service Number:	26471-62-5
Abbreviation:	TDI
Project Number:	152-EU-ENV

This study was sponsored by the International Isocyanate Ins. behalf of the following:

The Dow Chemical Company
ARCO Chemical
Bayer Corporation
BASF Corporation
ICI Americas, Inc.

Very truly yours,

M.J. Blankenship
Managing Director

A 04

HI Project 152

HI ref. 11298

Determination of the effect of TDI on the reproduction of Daphnia magna

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March 1998

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A 05 III Project 152

11298

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T/SE/MES/ECOTOXICOLOGIE

DETERMINATION OF THE EFFECT OF

TDI 80/20

ON THE REPRODUCTION OF *DAPHNIA MAGNA* ACCORDING TO OECD GUIDELINE N°211 (REVISED DRAFT DOCUMENT OF DECEMBER 1996) (Semi-static test)

Study D 322 report

The study was performed according to Good Laboratory Practice of the OECD

By :	Edith CERBELAUD	Study director
	Monique SAUGUES and Pierre CELLIER	Aquatic study technicians

With the participation of :

Michel ARGOUD	Breeding manager

I declare that the present study was performed under my control, according to the procedures described herein, and that this study report is an exact and reliable report of the results obtained.

Décines, on: December 16, 1997

The Study Director:

Edith CERBELAUD

E. Cerbelaud

This report comprises 15 pages and 2 appendixes.

A 06

Quality Assurance Statement

STUDY TITLE : Determination of the effect of:

TDI 80/20

on the reproduction of *Daphnia magna* according to OECD Guideline N°211 (revised draft document of December 1996) (Semi-static test)

STUDY REPORT **D 322**

Phase audited with date (Day/Month/Year)		Date of inspection report	Inspection report seen by : Study Director	Inspection report seen by : Installation Director
Protocol review	17/09/97	17/09/97	19/09/97	19/09/97
Experimental period	/	/	/	/
Final report	25/11/97	25/11/97	28/11/97	25/11/97

None of the audits performed during this study gave evidence of deviations with respect to GLP regulations which could jeopardize the quality or integrity of the study.

DATE: 12/12/ 1997

VISA QUALITY ASSURANCE :
Bernard MEYER

1 - GENERAL INFORMATION

1.1 Study sponsor and study monitor

Study sponsor: International Isocyanate Institute, inc.
201 Main Street, Suite 403
La Crosse
WISCONSIN, 54601 - U.S.A.

Study monitor: Arielle GARD
RHONE-POULENC CHIMIE, CH/EU/DI/RSP
85, avenue des frères Perret
BP 62
69192 SAINT-FONS CEDEX - FRANCE

1.2 Aim of the study

To assess the effect of **TDI 80/20** on the reproductive output of *Daphnia magna* (strain IRCHA - INERIS, BP 2, 60550 VERNEUIL EN HALATTE), cultivated in the laboratory of ECOTOXICOLOGY of CRIT-D.

The sensitivity of the *daphnia* was determined in study D 323 (see report in Appendix 1).

1.3 Characteristics of the test substance

- References: TDI 80/20 (also called SCURANATE T 80), containing about 80% of toluene 2,4-diisocyanate and 20% of toluene 2,6-diisocyanate, batch 97/24106, n° CAS : 26471-62-5, received in the laboratory in the 36th week of 1997.
- Origin: Rhône-Poulenc Chimie, Pont-de-Claix Factory, BP 17, 38800 Pont-de-Claix, France
- Place and conditions for storage: at room temperature, in the ventilated chamber (room 111, building M 26) reserved for test substances.
- Characteristics: at 20 °C, the test substance is a colourless or slightly yellow liquid, which readily hydrolyses in water to give polyureas, oligoureas, 2,4-diaminotoluene and 2,6-diaminotoluene.

1.4 Localisation of the study

RHONE-POULENC INDUSTRIALISATION - Laboratory of ECOTOXICOLOGY.

1.5 Time schedule

- Test solution preparation : on September 23, 1997
- Test : from September 24, 1997 to October 15, 1997

1.6 Localisation of study archives

In a cupboard reserved for G.L.P. studies, located in room 138, building M26.

2 - STUDY CONDUCT

2.1 Principle

To assess, in the conditions described in this report, the no observed effect concentration (NOEC), and the lowest observed effect concentration (LOEC), of the test substance on the reproductive output of *Daphnia magna*.

Female *daphnia* (the parent animals), aged less than 24 hours at the start of the test, are exposed to the test substance added to water at a range of concentrations. The test duration is 21 days. At the end of the test, the total number of living offspring produced per parent animal alive at the end of the test is assessed. The reproductive output of the animals exposed to the test substance is compared to that of the controls.

2.2 Reagents

2.2.1 Chemical reagents

All chemical reagents were of analytical grade. Water used for the preparation of solutions was double distilled.

2.2.2 Biological reagents

Daphnia magna Strauss 1820 (*Cladocera crustacea*) commonly called *daphnia* (strain IRCHA), obtained from the breeding of the laboratory of ECOTOXICOLOGY of CRIT-D.

At the start of the test, the *daphnia* were less than 24 hours old, and were not first brood progeny: the day before the test, only 2- to 3-week old *daphnia* were left in the culture; on the day of the test, the new born *daphnia* were collected.

Daphnia were sieved: the *daphnia* used, aged less then 24 hours, passed through a sieve of 560 µm mesh, and were retained on a sieve of 315 µm mesh.

A pre-test acclimation period to the test medium was not included.

2.2.3 Test medium

The test medium was Elendt M4 medium. Its composition was:

$CaCl_2$, 2 H_2O	293.8	mg/l	NaBr	0.016	mg/l
$MgSO_4$, 7 H_2O	123.3	mg/l	Na_2MoO_4, 2 H_2O	0.063	mg/l
$NaHCO_3$	64.8	mg/l	$CuCl_2$, 2 H_2O	0.0168	mg/l
KCl	5.8	mg/l	$ZnCl_2$	0.013	mg/l
Na_2SiO_3, 5 H_2O	7.465	mg/l	$CoCl_2$, 6 H_2O	0.010	mg/l
$NaNO_3$	1.370	mg/l	KI	0.0033	mg/l
KH_2PO_4	0.715	mg/l	SeO_2	0.0014	mg/l
K_2HPO_4	0.920	mg/l	NH_4VO_3	0.0006	mg/l
H_3BO_3	2.8595	mg/l	Na_2EDTA, 2 H_2O	2.5	mg/l
$MnCl_2$, 4 H_2O	0.3605	mg/l	$FeSO_4$, 7 H_2O	0.9955	mg/l
LiCl	0.306	mg/l	Thiamine, HCl	0.075	mg/l
RbCl	0.071	mg/l	Cyanocobalamine	0.001	mg/l
$SrCl_2$, 6 H_2O	0.152	mg/l	Biotine	0.0008	mg/l

Remark: The concentrations of $NaNO_3$, KH_2PO_4 and K_2HPO_4, were higher than in Elendt M4 medium. Validity criteria were met in the test (see § 3), results are thus considered valid.

2.2.4 Feeding

During the test, the *daphnia* were fed with a concentrated algal cell suspension. The concentrated algal cell suspension came from an algal culture of *Pseudokirchneriella subcapitata* cultivated in the ISO 8692 medium in the laboratory of ECOTOXICOLOGY. The culture was centrifuged, washed with double distilled water, then centrifuged and resuspended in double distilled water. Its carbon content was determined by optical density measurement ($OD_{665nm,1cm}$). The relationship between the total carbon (TC) and the optical density was: OD = 0.0083 TC + 0.00093, with r^2 = 99.98%.

2.3 Test solutions

The substance (**1.3**) and the test medium (**2.2.3**) were introduced in 10-liter plastic containers in the respective proportions of 0.28 mg, 0.55 mg, 1.1 mg, 2.2 mg and 4.4 mg per 1000 g. Nine litres of solution were prepared for each quantity of test substance.

The containers were closed. Their content were magnetically stirred (60-mm long cylindrical magnetic bar, about 100 rpm) during 24 hours at 18-22 °C at ± 1°C.

pH was measured after the 24 hours of stirring.

The solutions obtained were the test solutions. They were stored at about 4°C in the closed containers for the whole duration of the test.

The concentrations of the test solutions were expressed in mg of test substance added per kg of test medium.

At the end of the test, the test solutions were collected and discarded according to the procedures of CRIT-D.

2.4 Reference substance

Potassium dichromate ($K_2Cr_2O_7$), analytical grade (see D 323 study report in appendix 1).

2.5 Test containers

180 ml- glass serum bottles closed with chlorobutyl rubber septa. Containers and septa were machine washed with the following steps: acid washing (complex H_3PO_4), then detergent washing (Néodisher A8 : 20- 25 g by washing cycle), then rinsing twice with deionized water.

2.7 Test environment

The test was performed in the rooms reserved for this use, with regulated temperature. The temperature was measured once a week in a control test solution and in a 4.4 mg/kg test solution, in the fresh and the old test solutions at renewal. It varied between 20.6 °C and 21.0 °C.

These values were consistent with the temperatures (temperature between 18 °C and 22 °C at ± 1 °C) required by the method.

Photoperiod was 16 hours light with an intensity of about 1000 lux at the surface of the test solutions.

2.8 Study design

2.8.1 Sensitivity of biological reagent

The *daphnia* had a 24 h- EC 50 with potassium dichromate of 0.99 ppm (weight/weight) (see D 323 study report in appendix 1).

2.8.2 Test procedure

On September 24, 1997, ten test containers (**2.5**) were prepared for each test solution, and 50 ml of test solution (**2.3**) were introduced in each test container. Fifteen test containers (**2.5**) were prepared for the controls, and 50 ml of test medium (**2.2.3**) were introduced in each test container.

One *daphnia* aged less than 24 hours was introduced in each test container at the beginning of the test, as well as the first algal ration. The test containers were closed.

Every Mondays, Wednesdays and Fridays, the test solutions were renewed, and the offspring produced counted: the parent animals were transferred in the fresh test solutions, and the young *daphnia* were counted in the old test solutions. Mortality among parent animals were recorded five times per week.

A concentrated algal cell suspension (**2.2.4**) was supplied five times per week in each test container, at a level of 0.2 mg of organic carbon per day and per *daphnia* on Mondays, Tuesdays, Wednesdays and Thursdays, and 0.4 mg of organic carbon per day and per *daphnia* on Fridays. When the test solutions were renewed, the algal cell suspension was supplied after the test solution renewal.

2.8.3 Analysis and measurements

The test substance readily hydrolyses to give polyureas, oligoureas, 2,4-diaminotoluene and 2,6-diaminotoluene.

Analysis of 2,4-diaminotoluene and 2,6-diaminotoluene were performed by the laboratory CP/MAN of Rhône-Poulenc Industrialisation (see report in appendix 2).

These analysis were performed one time per week, for each test solution, on the fresh test solutions, and on the same test solutions after *daphnia* exposure, at renewal.

The dissolved oxygen concentration, the temperature, and the pH were measured one time per week, on the fresh control test solutions and on the most concentrated fresh test solutions, and on the same test solutions after *daphnia* exposure, at renewal.

3 - RESULTS

Records of living offspring by each parent animal, deaths among parent animals, pH, dissolved oxygen, temperature and hardness are reported in tables n° 2 to n° 7.

Results of analysis of the test solutions are reported in table n° 8.

The test is considered valid as the following criteria were met in the controls:

- The mortality of the parent animals was ≤ 20 % at the end of the test.
- The mean number of live offspring produced per parent animal surviving at the end of the test was ≥ 60.

The coefficient of variation for control fecundity was 21 %.

NOEC and LOEC were determined by a multiple comparison procedure (Dunnett's procedure) with a confidence level of 95 %. In addition, the concentration that causes a 50 % reduction in reproductive output (EC50) and its 95 % confidence interval were calculated using a regression model. Results are reported in table n° 1 below (see § 4).

4 - CONCLUSION

Organism	*Daphnia magna*
Substance	TDI 80/20
NOEC*	1.1 mg/kg
LOEC*	2.2 mg/kg
EC50* *(95 % confidence interval)*	2.0 mg/kg *(1.7 mg/kg - 2.3 mg/kg)*

** on daphnia reproduction, in mg/kg of the initial quantity of TDI 80/20 in the test solution*

REMARK

Analysis, performed by the laboratory CP/MAN of Rhône-Poulenc Industrialisation (report in appendix 2 and table n°8), has shown that:

- *conversion of TDI to TDA was incomplete and was shown to be between 40 and 48 % (in weight) and between 57 and 69 % (in mole) in the initial test solutions at the beginning of the test,*
- *TDA concentrations in the test solutions decreased during the last week of exposure, this decrease was 20 % in the 1.1 mg/kg test solution which corresponds to the NOEC,*
- *TDA concentrations during exposure were stable in most cases.*

Table n° 2:
Living offspring, deaths of adults and measurements for the controls

Organism : *Daphnia magna*
Substance : TDI 80/20
Method : OECD n° 211 (revised draft of December 1996)

Day	0	2	5	7	9	12	14	16	19	21	
Medium renewal	yes	yes	yes	yes	yes	yes	yes	yes	yes	-	
Hardness (mg/l as $CaCO_3$)	267	-	-	-	-	-	-	-	-	-	
pH											
new test solution	8.1	-	-	8.3	-	-	8.2	-	-	-	
old test solution	-	-	-	8.9	-	-	8.8	-	-	8.7	
O2 (% saturation)											
new test solution	96	-	-	100	-	-	95	-	-	-	
old test solution	-	-	-	106	-	-	103	-	-	100	
Temperature (°C)											
new test solution	20.8	-	-	20.6	-	-	20.8	-	-	-	
old test solution	-	-	-	20.6	-	-	20.8	-	-	21	
No. live offspring											total
Container n°1	-	0	0	0	7	12	20	0	11	3	53
Container n°2	-	0	0	0	7	9	20	0	15	16	67
Container n°3	-	0	0	0	10	14	0	18	13	18	73
Container n°4	-	0	0	0	1	15	23	0	16	12	67
Container n°5	-	0	0	0	0	1	22	3	11 D	-	-
Container n°6	-	0	0	0	0	8	20	15	19	0	62
Container n°7	-	0	0	0	3	11	23	0	11	16	64
Container n°8	-	0	0	0	9	12	23	0	16	23	83
Container n°9	-	0	0	0	0	10	4	0	8	11	33
Container n°10	-	0	0	0	0	8	0	13	15	15	51
Container n°11	-	0	0	0	7	9	19	0	16	24	75
Container n°12	-	0	0	0	11	14	19	0	18	24	86
Container n°13	-	0	0	0	8	12	14	0	10	17	61
Container n°14	-	0	0	0	4	9	20	0	13	20	66
Container n° 5	-	0	0	0	1	12	20	10	13	0	56
Total*											897
Cumulative adult mortality											1

D: death of adult
** Total number of living offspring produced by parent animals alive at the end of the test*

Table n° 3:
Living offspring, deaths of adults and measurements for the 0.23 mg/kg test solution

Organism : *Daphnia magna*
Substance : TDI 80/20
Method : OECD n° 211 (revised draft of December 1996)

Day	0	2	5	7	9	12	14		19	21	
Medium renewal	yes	yes	yes	yes	yes	yes	yes	yes	yes	-	
Hardness (mg/l as $CaCO_3$)	262	-	-	-	-	-	-	-	-	-	
pH											
new test solution	8.0	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
O2 (% saturation)											
new test solution	-	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
Temperature (°C)											
new test solution	-	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
No. live offspring											total
Container n°1	-	0	0	0	12	14	0	18	9	15	68
Container n°2	-	0	0	0	6	9	15	0	9	16	55
Container n°3	-	0	0	0	10	11	22	8	7	16	74
Container n°4	-	0	0	0	0	12	16	9	11	6	54
Container n°5	-	0	0	0	1	14	22	17	14	0	68
Container n°6	-	0	0	0	5	18	19	2	11	15	70
Container n°7	-	0	0	0	0	13	18	15	16	0	62
Container n°8	-	0	0	0	11	11	1 D	-	-	-	-
Container n°9	-	0	0	0	0	13	16	13	9	5	56
Container n°10	-	0	0	0	10	11	1	14	15	20	71
Total*											578
Cumulative adult mortality											1

D: death of adult
** Total number of living offspring produced by parent animals alive at the end of the test*

Table n° 4:
Living offspring, deaths of adults and measurements for the 0.55 mg/kg test solution

Organism : *Daphnia magna*
Substance : TDI 80/20
Method : OECD n° 211 (revised draft of December 1996)

Day	0	2	5	7	9	12	14	16	19	21	
Medium renewal	yes	yes	yes	yes	yes	yes	yes	yes	yes	-	
Hardness (mg/l as $CaCO_3$)	262	-	-	-	-	-	-	-	-	-	
pH											
new test solution	8.0	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
O2 (% saturation)											
new test solution	-	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
Temperature (°C)											
new test solution	-	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
No. live offspring											total
Container n°1	-	0	0	0	7	9	19	0	13	14	62
Container n°2	-	0	0	0	9	11	17	3	14	15	69
Container n°3	-	0	0	0	8	6	3	0	0	0	17
Container n°4	-	0	0	0	0	12	20	13	8	0	53
Container n°5	-	0	0	0	0	13	18	12	15	0	58
Container n°6	-	0	0	0	12	14	22	0	14	22	84
Container n°7	-	0	0	0	9	16	0	23	18	17	83
Container n°8	-	0	0	0	8	10	16	13	2	15	64
Container n°9	-	0	0	0	9	14	16	0	14	14	67
Container n°10	-	0	0	0	0	13	18	11	18	1	61
Total*											618
Cumulative adult mortality											0

D: death of adult
** Total number of living offspring produced by parent animals alive at the end of the test*

Table n° 5:
Living offspring, deaths of adults and measurements for the 1.1 mg/kg test solution

Organism : *Daphnia magna*
Substance : TDI 80/20
Method : OECD n° 211 (revised draft of December 1996)

Day	0	2	5	7	9	12	14	16	19	21	
Medium renewal	yes	yes	yes	yes	yes	yes	yes	yes	yes	-	
Hardness (mg/l as $CaCO_3$)	281	-	-	-	-	-	-	-	-	-	
pH											
new test solution	8.1	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
O2 (% saturation)											
new test solution	-	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
Temperature (°C)											
new test solution	-	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
No. live offspring											total
Container n°1	-	0	0	0	8	16	1	13	15	17 D	-
Container n°2	-	0	0	0	0	13	22	11	0	11	57
Container n°3	-	0	0	0	9	18	0	3 D	-	-	-
Container n°4	-	0	0	0	10	16	19	15	15	3	78
Container n°5	-	0	0	0	8	13	19	0	14	14	68
Container n°6	-	0	0	0	0	11	19	15	16	0	61
Container n°7	-	0	0	0	1	12	18	11	1	10	53
Container n°8	-	0	0	0	6	14	0	15	11	14	60
Container n°9	-	0	0	0	8	10	16	10	16	0	60
Container n°10	-	0	0	0	0	12	21	12	21	0	66
Total*											503
Cumulative adult mortality											2

D: death of adult
** Total number of living offspring produced by parent animals alive at the end of the test*

Table n° 6:
Living offspring, deaths of adults and measurements for the 2.2 mg/kg test solution

Organism : *Daphnia magna*
Substance : TDI 80/20
Method : OECD n° 211 (revised draft of December 1996)

Day	0	2	5	7	9	12	14	16	19	21	
Medium renewal	yes	yes	yes	yes	yes	yes	yes	yes	yes	-	
Hardness (mg/l as $CaCO_3$)	263	-	-	-	-	-	-	-	-	-	
pH											
new test solution	8.1	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
O2 (% saturation)											
new test solution	-	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
Temperature (°C)											
new test solution	-	-	-	-	-	-	-	-	-	-	
old test solution	-	-	-	-	-	-	-	-	-	-	
No. live offspring											total
Container n°1	-	0	0	0	1	2	15	0	11	15	44
Container n°2	-	0	0	0	0 7 AE	2 2 AE	9 1 AE	0	4 1 AE	6 3 AE	21 14 AE
Container n°3	-	0	0	0	0	1 7 AE	0	0 5 AE	1 3 AE	0	2 15 AE
Container n°4	-	0	0	0	2 5 AE	4 1 AE	14	0	11	1 6 AE	32 12 AE
Container n°5	-	0	0	0	0 5 AE	7 2 AE	6 D	-	-	-	-
Container n°6	-	0	0	0	0 5 AE	1 8 AE	10 1 AE	1	10	4	26 14 AE
Container n°7	-	0	0	0	0	4 3 AE	4	14	15	5 1 AE	42 4 AE
Container n°8	-	0	0	0	0	0 7 AE	7	13	0	0 5 AE	20 12 AE
Container n°9	-	0	0	0	2 5 AE	0 7 AE	14	0	4	12	32 12 AE
Container n°10	-	0	0	0	0 2 AE	0	2	2	5 3 AE	0	9 5 AE
Total*											228
Cumulative adult mortality											1

D: death of adult
AE: Aborted eggs
** Total number of living offspring produced by parent animals alive at the end of the test*

Table n° 7:
Living offspring, deaths of adults and measurements for the 4,4 mg/kg test solution

Organism : *Daphnia magna*
Substance : TDI 80/20
Method : OECD n° 211 (revised draft of December 1996)

Day	0	2	5	7	9	12	14	16	19	21	
Medium renewal	yes	yes	yes	yes	yes	yes	yes	yes	yes	-	
Hardness (mg/l as $CaCO_3$)	260	-	-	-	-	-	-	-	-	-	
pH											
new test solution	8.1	-	-	7.7	-	-	7.6	-	-	-	
old test solution	-	-	-	8.8	-	-	8.5	-	-	8.7	
O2 (% saturation)											
new test solution	100	-	-	92	-	-	97	-	-	-	
old test solution	-	-	-	116	-	-	107	-	-	100	
Temperature (°C)											
new test solution	20.8	-	-	20.6	-	-	21	-	-	-	
old test solution	-	-	-	20.6	-	-	21	-	-	21	
No. live offspring											total
Container n°1	-	0	0	0	0	0 4 AE	0 16 AE	0	0 4 AE	8 3 AE	8 27 AE
Container n°2	-	0	0	0	0	4 4 AE	0 14 AE	0 14 AE	0 3 AE	0	4 35 AE
Container n°3	-	0	0	0	0	0	0 14 AE	0 15 AE	1 9 AE	0	1 38 AE
Container n°4	-	0	0	0	0	0	0 11 AE	0 3 AE	0	0 2 AE	0 16 AE
Container n°5	-	0	0	0	0	0 8 AE	0 13 AE	0	0 10 AE	1 13 AE	1 44 AE
Container n°6	-	0	0	0	0	0	0 20 AE	0 17 AE	2 15 AE	0	2 52 AE
Container n°7	-	0	0 D	-	-	-	-	-	-	-	-
Container n°8	-	0	0	0	0	1 8 AE	0 18 AE	0	2 4 AE	9 3 AE	12 33 AE
Container n°9	-	0	0	0	0	0	0 1 AE	9	0	0 14 AE	9 15 AE
Container n°10	-	0	0	0	0	0 7 AE	0	12	1 7 AE	0 5 AE	13 19 AE
Total*											50
Adult mortality											1

D: death of adult
AE: Aborted eggs
** Total number of living offspring produced by parent animals alive at the end of the test*

Table n° 7:
Results of chemical analysis of (2,4-diaminotoluene + 2,6-diaminotoluene)
(see report in appendix 2)

Organism : *Daphnia magna*
Substance : TDI 80/20
Method : OECD n° 211 (revised draft of December 1996)

Initial quantity of substance (mg/kg)	2,4-diaminotoluene + 2,6-diaminotoluene (mg/l)							
	Week 1 sample		Week 2 sample		Week 3 sample		Week 4 sample	
	Fresh (97/09/24)	Old (97/09/26)	Fresh (97/09/29)	Old (97/10/01)	Fresh (97/10/06)	Old (97/10/08)	Fresh (97/10/13)	Old (97/10/15)
0.28	0.132	0.132	0.139	0.110	0.132	0.101	0.092	0.093
sample reference	MSAD322 05	MSAD322 10	MSAD322 16	MSAD322 21	MSAD322 26	MSAD322 31	MSAD322 33	MSAD322 38
0.55	0.218	0.214	0.225	0.195	0.210	0.180	0.160	0.167
sample reference	MSAD322 04	MSAD322 09	MSAD322 15	MSAD322 20	MSAD322 25	MSAD322 30	MSAD322 32	MSAD322 37
1.1	0.501	0.476	0.496	0.455	0.484	0.437	0.403	0.372
sample reference	MSAD322 03	MSAD322 08	MSAD322 14	MSAD322 19	MSAD322 24	MSAD322 29	MSAD322 31	MSAD322 36
2.2	1.062	1.062	1.091	1.011	1.037	1.024	0.990	0.892
sample reference	MSAD322 02	MSAD322 07	MSAD322 13	MSAD322 18	MSAD322 23	MSAD322 28	MSAD322 30	MSAD322 35
4.4	1.814	1.788	1.871	1.779	1.800	1.728	1.798	1.621
sample reference	MSAD322 01	MSAD322 06	MSAD322 12	MSAD322 17	MSAD322 22	MSAD322 27	MSAD322 29	MSAD322 34

Copy of the analysis certificate given by the sponsor
(not performed according to G.L.P.)

TDI T80, coulage C 703

Lot 97 / 24106

Analyse chomato

Composants	ppm
chlore hydrolysable(moyenne)	40
o DCB	<10
impuretés légères	85
M1 + M2	<10
lourd A	35
lourd B	20
lourd C	<100
lourd E	<100
lourd F	<100
lourd G	<100
lourd J	<100

B 06

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APPENDIX 1 TO REPORT D 322

DETERMINATION OF THE ACUTE ECOTOXICITY AFTER 24 HOURS OF EXPOSURE

POTASSIUM DICHROMATE (reference substance)

ON *DAPHNIA MAGNA* ACCORDING TO METHOD C.2. PUBLISHED IN THE OFFICIAL JOURNAL OF EUROPEAN COMMUNITIES OF 29.12.92

Study D 323 report

The study was performed according to Good Laboratory Practice of the O.E.C.D.

By :	Edith CERBELAUD	Study director
	Monique SAUGUES	Aquatic study technicians

With the participation of:

Michel ARGOUD	breeding manager

I declare that the present study was performed under my control, according to the procedures described herein, and that this study report is an exact and reliable report of the results obtained.

Décines, on: December 16, 1997

The Study Director:

Edith CERBELAUD

E. Cerbelaud

1 - GENERAL INFORMATION

1.1 Study sponsor

Laboratoire ECOTOXICOLOGIE Service Méthodes et Essais
RHONE-POULENC INDUSTRIALISATION

1.2 Aim of the study

To determine the sensitivity of the *daphnia* (*Daphnia magna*, strain IRCHA - INERIS, BP 2, 60550 VERNEUIL EN HALATTE) obtained on September 24,1997, from the laboratory of ECOTOXICOLOGY of CRIT-D L / evaluation of the acute toxicity of test compound: potassium dichromate.

1.3 Test substance

Potassium dichromate, RP NORMAPUR grade, from PROLABO, FRANCE, (batch n° 90002), stored at room temperature in the laboratory.

1.4 Time schedule :

Test : began on September 24, 1997

: ended on September 25, 1997

1.5 Localisation of study archives

In a cupboard reserved for G.L.P. studies, located in room 138, building M 26.

2 - STUDY CONDUCT

2.1 Principle

Determination of the initial concentration of the test compound which, after 24 hours, is inhibitory to the mobility for 50 % of the *daphnia* in the test.

This concentration measured in ppm (w/w) is the initial median effective concentration of test compound to which 50 % of test organisms are immobilised (24 hour- EC 50).

The study was conducted in one step.

The 24 hour- EC 50 for potassium dichromate should be between 0.6 and 1.7 ppm (w/w).

2.2 Reagents

2.2.1 Chemical reagents

All chemical reagents were of analytical grade. Water used for the preparation of solutions was double distilled.

2.2.2 Biological reagents

Daphnia magna Strauss 1820 (*Cladocera Crustacea*) commonly called *daphnia* (strain IRCHA), obtained from the breeding of the laboratory of ECOTOXICOLOGY of CRIT-D.

The *daphnia* used passed through a sieve of 560 µm mesh, and were retained on a sieve of 315 µm mesh.

2.2.3 Test medium

The test medium was prepared with double distilled water with conductivity of: 1.2 $\mu S.cm^{-1}$.

Its composition was:

$CaCl_2$. 2 H_2O	0.294 g/l
$MgSO_4$. 7 H_2O	0.12325 g/l
$NaHCO_3$	0.06475 g/l
KCl	0.00575 g/l

The test medium was aerated with compressed air. Just before its use for the test, its pH was 8.0 at 20 °C and its oxygen concentration 100 % saturation.

2.3 Test solutions

The initial solution of potassium dichromate was prepared at a concentration of 100 ppm (w/w) in double distilled water.

A solution at a concentration of 10 ppm (w/w) was obtained by a 10 fold dilution of the initial solution in the test medium.

The test solutions were obtained by dilution of the 10 ppm solution in the test medium.

At the end of the test, the test solutions were discarded directly with the effluents of CRIT-D.

2.4 Test containers

The test was performed in 100-ml pyrex glass beakers.

2.5 Test environment

The test was performed in the room reserved for this use, with regulated temperature. The temperature varied between 19.8 and 20.0 °C during the test.

These values were consistent with the temperatures (between 18 °C and 22 °C at ± 1 °C) required by the method.

Daphnia exposure was performed in the dark.

2.6 Study design

9 test solutions (2.3) of the test substance (1.3) were carried out at a concentration of 0.44, 0.60, 0.80, 0.94, 1.1, 1.4, 1.7, 2.1 and 2.5 ppm (w/w).

On September 24, 1997, in 1 test container (2.4) per concentration, 40 g of each test solution (2.3) were introduced. One additional container was filled with 40 g of test medium (2.2.3)(control).

Twenty *daphnia* were added to each container (in 15 minutes for all the containers).

24 hours after having introduced all *daphnia*, on September 25, 1997, immobilised *daphnia* were counted in each test container.

Just after counting, the dissolved oxygen concentration was measured in the test solution corresponding to the smallest concentration to which 100 % of *daphnia* were immobilised.

3 - RESULTS

3.1 Physico-chemical parameters and percentage *daphnia* immobilisation

Physico-chemical parameters measured and the percentage *daphnia* immobilisation obtained are reported in table n° 1.

3.2 Calculation of 24 hour- EC 50

The 24 hour- EC 50 of potassium dichromate and the 95 % confidence limits were calculated by the probit method (Statgraphics software).

The value found with two significant figures was :

24 hour- EC 50	**0.99 ppm** (w/w)

The 95 % confidence limits were 0.88 ppm and 1.2 ppm (w/w).

4 - CONCLUSION

The 24 hour- EC 50 of potassium dichromate (reference substance) on *daphnia* (*Daphnia magna*) obtained on September 24, 1997, from the breeding of the laboratory of ECOTOXICOLOGY, was 0.99 ppm (w/w).

This value is within the limit values of 0.6 ppm and 1.7 ppm (w/w) (values considered valid by the laboratory).

table n° 1:
Physico-chemical parameters and % *daphnia* immobilisation

Organism : *Daphnia magna*
Substance : Potassium dichromate
Method : Method C.2. published in the Official Journal of European Communities of 29.12.92

Concentrations in ppm (w/w)	Immobilisation		O_2
	number/20	%	%
Control	0	0	-
0.44	0	0	
0.60	2	10	-
0.80	5	25	-
0.94	8	40	-
1.1	11	55	-
1.4	15	75	-
1.7	20	100	94
2.1	20	100	-
2.5	20	100	-

B 11

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November 24th, 1997

Ref : CP/MAN/338/97/0459/DSI/mg - Study 97 006 ATO

From :

D. SILVESTRE CP/MAN

To :

E. CERBELAUD T/SE/MES

DETERMINATION OF THE EFFECT OF TDI 80/20 ON THE REPRODUCTION OF DAPHNIA MAGNA ACCORDING TO OECD GUIDELINE N° 211 (STUDY D 322)

2,4-TDA and 2,6-TDA Determination in Study D 322 Test Solutions

Study Report

Organic Trace Analysis Laboratory

Study number : 97 006 ATO

Scientist : D. LEGAT
Study Director : D. SILVESTRE

Study 97 006 ATO - Study Information

Title

2,4-TDA and 2,6-TDA Determination in Study D322 Test Solutions.

Sponsor

Edith CERBELAUD
Rhône-Poulenc Industrialisation - CRIT/D
24 avenue Jean Jaurès
69153 DECINES CHARPIEU CEDEX - FRANCE

Study Director

Dr. Dominique SILVESTRE

Contributing Scientist

Daniel LEGAT

Performing Laboratory

Organic Trace Analysis Laboratory
Rhône-Poulenc Industrialisation - CRIT/D
24 avenue Jean Jaurès
69153 DECINES - CHARPIEU CEDEX - FRANCE

Experimental dates

September 1997 - October 1997

GLP Statement

I, the undersigned, hereby declare that Study 97 006 ATO meets the requirements of the OECD Good Laboratory Practice in the testing of Chemicals. This report is an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the results.

Signature :  Date : 12/12/97

Quality Assurance Statement

STUDY TITLE : Determination of the effect of:

TDI 80/20

on the reproduction of *Daphnia magna* according to OECD Guideline N°211 (STUDY D 322)

2,4-TDA and **2,6-TDA** Determination in Study D 322 Test Solutions.

STUDY REPORT **97 006 ATO**

Phase audited with date (Day/Month/Year)		Date of inspection report	Inspection report seen by : Study Director	Inspection report seen by : installation Director
Protocol review	/	/	/	/
Experimental period	/	/	/	/
Final report	11/12/97	11/12/97	12/12/97	12/12/97

None of the audits performed during this study gave evidence of deviations with respect to GLP regulations which could jeopardize the quality or integrity of the study.

DATE: 12 /12/ 1997

VISA QUALITY ASSURANCE :
Bernard MEYER

Table of Contents

1. Subject

Determination of 2,4-TDA and 2,6-TDA in the test solutions of Study D322 by High Performance Liquid Chromatography.

2. Standards

Standard	Supplier	Purity	Lab. reference
2,4-Diaminotoluene (2,4-TDA)	ALDRICH 10,191-5	98 %	219/1
2,5-Diaminotoluene (2,6-TDA)	ALDRICH 14,811-3	97 %	220/1

3. Reagents

Reagent	Quality	Supplier
Water	Alpha Q	MILLIPORE
Acetonitrile (ACN)	HPLC	SDS
Ammonium Acetate	NORMAPUR	PROLABO

4. Equipment

- Volumetric tapered bottom flasks
- Pipets
- Syringes
- Analytical balance, METTLER AE 240

5. HPLC instrument

- Pump : SP 8800
- Injector : SP 8880
- Detector : UV, SHIMADZU SPD 10 AV
- Integrator : NELSON PE 1020

6. Analytical conditions

- Column : PARTISIL ODS2 250 mm x 4,6 mm
- Oven temperature : 25 °C
- Mobile phase : ACN / aqueous solution of ammonium acetate 0.2 g/l 70/30 V/V
- Flow rate : 1 ml/min.
- Injection volume : 20 ul
- Detection wavelength : 230 nm

7. Calibration

7.1 Standard solution preparation

7.1.1 Stock solutions

A 1010 mg/l stock solution of 2,4-TDA was prepared with 50.5 mg of 2,4-TDA weighed in a 50 ml tappered bottom flask, dissolved and diluted in a 1/1 mixture of ACN/water.

A 1004 mg/l stock solution of 2,6-TDA was similary prepared with 50.2 mg of 2,6-TDA.

7.1.2 Working standard solutions

Working standard solutions were prepared by means of serial dilutions of the stock solutions. The composition of the working standard solutions is given in the following table :

Standard solution reference	2,4-TDA concentration (mg/l)	2,6-TDA concentration (mg/l)
A1	5.05	5.02
A2	1.01	1.00
A3	0.50	0.50
A4	0.10	0.10

7.2 Calibration curve

Each standard solution was injected twice. An example of chromatogram corresponding to standard solution A2 is shown in Appendix 1.

The calibration curve is shown in Appendix 2.

8. Analytical Method Performance

8.1 Linearity

The linearity of the analytical method was verified within the range 0.1-5 mg/l.

8.2 Detection limit

2,4-TDA : 0.01 mg/l.

2,6-TDA : 0.01 mg/l.

Appendix 3 shows a chromatogram corresponding to a 2,4-TDA and 2,6-TDA 0.01 mg/l standard solution.

8.3 Repeatability

A sample (MSA - D322 - 17) was injected ten times.

The results obtained are the following :

Injection	2,4-TDA	2,6-TDA	2,4-TDA + 2,6-TDA
1	1.367	0.416	1.783
2	1.352	0.410	1.762
3	1.359	0.413	1.772
4	1.363	0.411	1.774
5	1.356	0.413	1.769
6	1.349	0.413	1.762
7	1.339	0.412	1.751
8	1.320	0.413	1.733
9	1.302	0.412	1.714
10	1.282	0.410	1.692
Mean Value	1.339	0.412	1.751
Standard Deviation	0.0285	0.0018	0.0293
Relative Standard Deviation	2.13 %	0.43 %	1.67 %

9. Results

Each sample was analyzed twice. The results are averaged in the following tables.

Date : 24/09/97

Sample	2.4-TDA	2.6-TDA	2.4-TDA + 2.6-TDA
MSA D 322 01	1.389	0.425	1.814
MSA D 322 02	0.821	0.241	1.062
MSA D 322 03	0.379	0.122	0.501
MSA D 322 04	0.164	0.054	0.218
MSA D 322 05	0.098	0.034	0.132

Date : 26/09/97

Sample	2.4-TDA	2.6-TDA	2.4-TDA + 2.6-TDA
MSA D 322 06	1.379	0.409	1.788
MSA D 322 07	0.826	0.236	1.062
MSA D 322 08	0.361	0.115	0.476
MSA D 322 09	0.165	0.049	0.214
MSA D 322 10	0.100	0.032	0.132
MSA D 322 11	nd < 0.01	nd < 0.01	-

Date : 29/09/97

Sample	2.4-TDA	2.6-TDA	2.4-TDA + 2.6-TDA
MSA D 322 12	1.443	0.428	1.871
MSA D 322 13	0.848	0.243	1.091
MSA D 322 14	0.376	0.120	0.496
MSA D 322 15	0.175	0.050	0.225
MSA D 322 16	0.106	0.033	0.139

Date : 01/10/97

Sample	2.4-TDA	2.6-TDA	2.4-TDA + 2.6-TDA
MSA D 322 17	1.366	0.413	1.779
MSA D 322 18	0.776	0.235	1.011
MSA D 322 19	0.340	0.115	0.455
MSA D 322 20	0.150	0.049	0.199
MSA D 322 21	0.079	0.031	0.110

Date : 6/10/97

Sample	2,4-TDA	2,6-TDA	2,4-TDA + 2,6-TDA
MSA D 322 22	1.384	0.416	1.800
MSA D 322 23	0.802	0.235	1.037
MSA D 322 24*	0.370	0.114	0.484
MSA D 322 25*	0.160	0.050	0.210
MSA D 322 26	0.096	0.035	0.132

* test 168 DLT 97 (analyses run on 7/10/97)

Date : 8/10/97

Sample	2,4-TDA	2,6-TDA	2,4-TDA + 2,6-TDA
MSA D 322 27	1.311	0.417	1.728
MSA D 322 28	0.789	0.235	1.024
MSA D 322 29	0.323	0.114	0.437
MSA D 322 30	0.134	0.046	0.180
MSA D 322 31	0.072	0.029	0.101

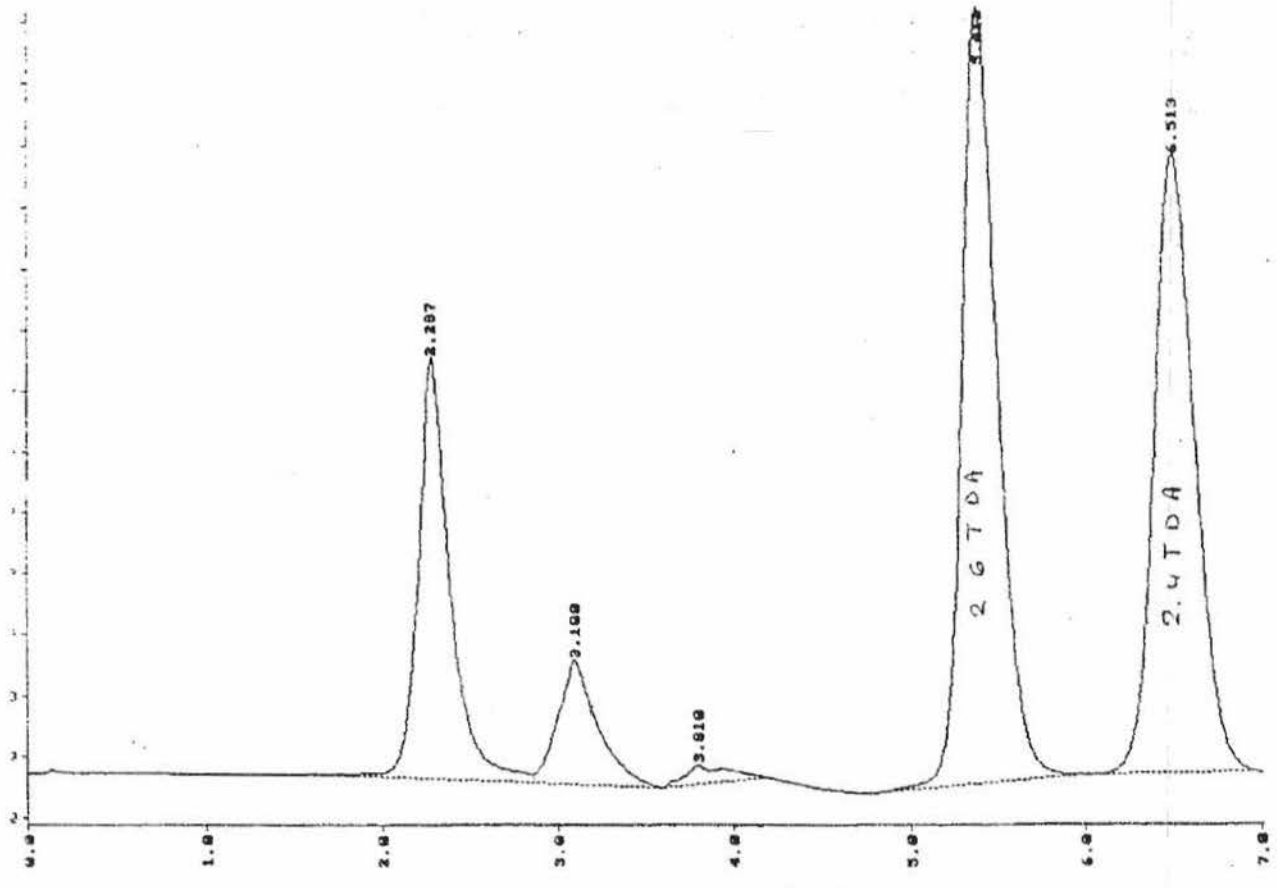
Date : 14/10/97

Sample	2,4-TDA	2,6-TDA	2,4-TDA + 2,6-TDA
MSA D 322 29	1.377	0.421	1.798
MSA D 322 30	0.753	0.237	0.990
MSA D 322 31	0.289	0.114	0.403
MSA D 322 32	0.112	0.048	0.160
MSA D 322 33	0.061	0.031	0.092

Date : 15/10/97

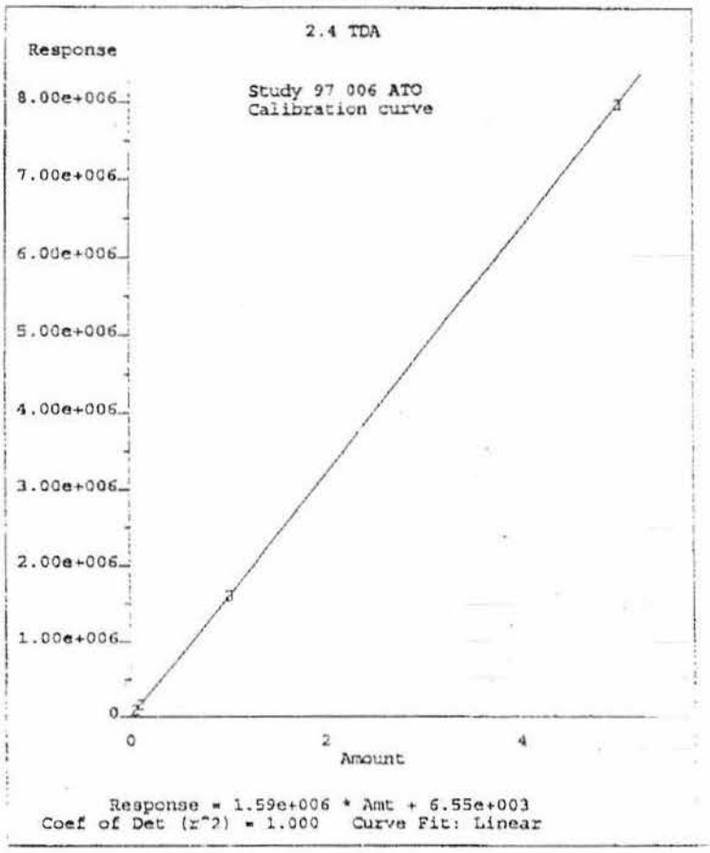
Sample	2,4-TDA	2,6-TDA	2,4-TDA + 2,6-TDA
MSA D 322 34	1.200	0.421	1.621
MSA D 322 35	0.656	0.236	0.892
MSA D 322 36	0.258	0.114	0.372
MSA D 322 37	0.115	0.052	0.167
MSA D 322 38	0.062	0.032	0.093

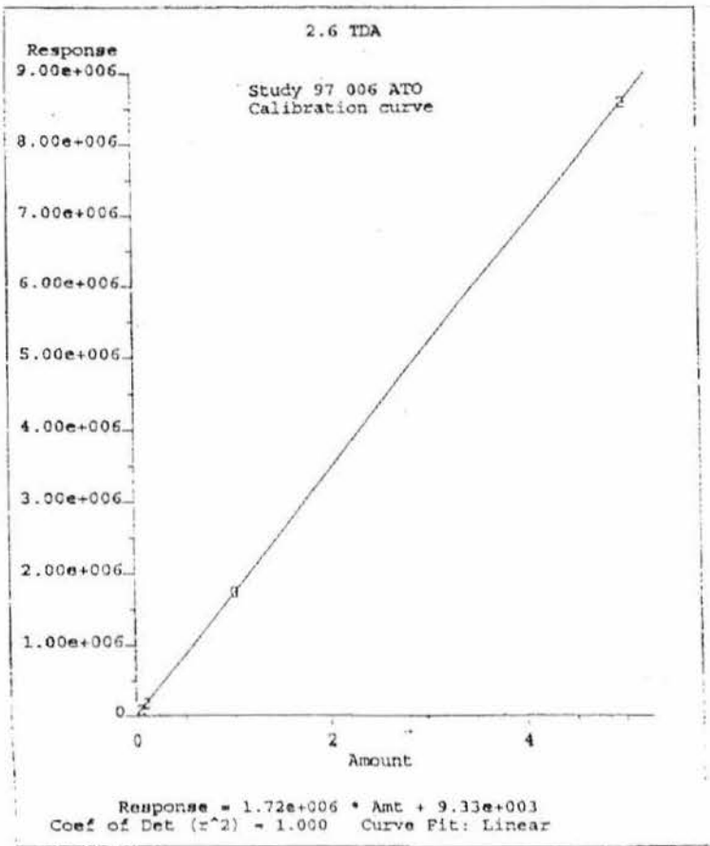
Appendix 1
Example of chromatogram : Standard Solution A2



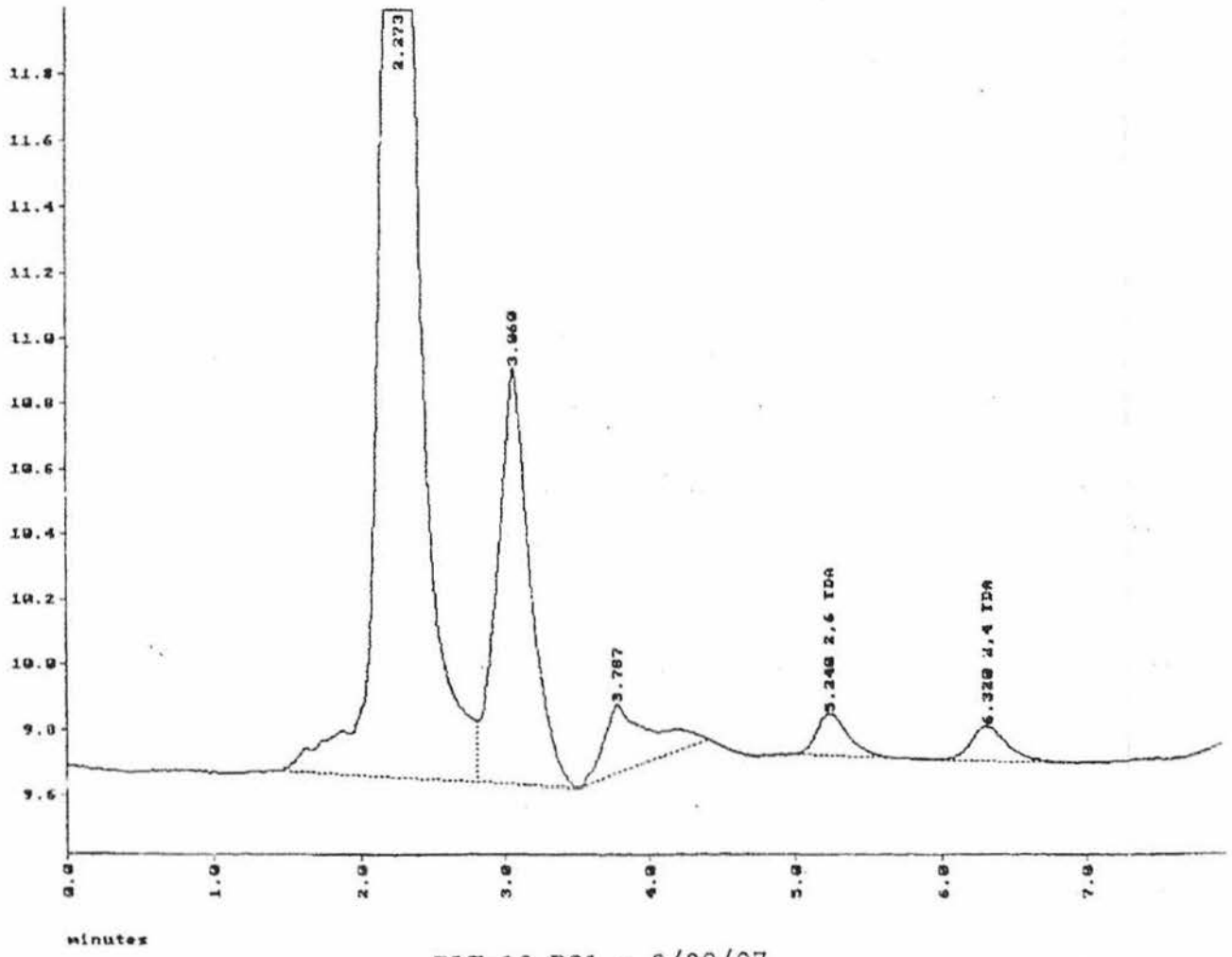
Q-RUN-02-D01 - 24/09/97
Study 97 006 ATO - solution A2

Appendix 2
Calibration Curves





Appendix 3
Chromatogram of a 0.01 mg/l 2,4-TDA
and 0.01 mg/l 2,6-TDA Standard Solution



DLT-18.D01 - 8/09/97
Study 97 006 ATO - solution S1

C 09

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